

S/N 09/804866

**Amendments to the Specification:**

Please insert the following new paragraph at the beginning of the specification before the "FIELD OF THE INVENTION" heading:

This application claims priority under 35 U.S.C. § 119 (e) to U.S. provisional patent application 60/193,208 filed March 30, 2000.

Please replace the paragraph beginning at p. 8, line 22 with the following paragraph:

Accordingly, in order to obtain sequence information, product-ion spectra of the  $[M+Ag]^+$  ion collected under several  $E_{cm}$  (for examples 1.5, 2.0, 2.5, and 3.0 eV, although other values may be used, values are chosen to provide as wide a range of abundant product ions as possible) and are summed to yield a composite spectrum. Such spectra exhibit a wide range of abundant product ions. Alternatively, product-ion spectra are acquired with an  $m/z$  dependent  $E_{cm}$  function (typically linear) to maximize the number of observable sequence-relevant product ions. The peak abundance file of the spectrum for sequencing, after elimination of peaks below a certain user-defined threshold, typically 10%, are read into a custom search algorithm written in Visual Basic (see FIG. 7) which looks for the triplet peak pattern of  $(m/z)_1$ ,  $(m/z)_2=(m/z)_1-18.0$ , and  $(m/z)_3=(m/z)_2-28.0$  as well as the doublet pattern of  $(m/z)_2$  and  $(m/z)_3$ , all to within  $\pm 0.5 m/z$  unit. Triplet and doublet peak patterns that are found are imported into any commercially available spreadsheet program, for example, Excel. A user-defined threshold is used to filter out noise. This user-defined threshold can be from 1 to 20% of maximum however, a preferred threshold is 10% of maximum. The differences in  $m/z$  values of neighboring triplets are used to search for cleaved amino acid residues or combinations of them by means of AminoCal, a shareware available on the World Wide Web (<http://www.protana.com/software/default.asp>) (<http://www.protana.com/software/default.asp>). The use of the spreadsheet program and AminoCal facilitates the sequence assignment, but is in no way crucial to this invention. For peptides that have C-terminal lysine and arginine residues, such as tryptic peptides, their

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product-ion spectra also contain prominent  $[y_n+H+Ag]^+$  ions that may be used to confirm residues cleaved from the C terminus. A  $m/z$  value of the  $[b_n-H+Ag]^+$  ion and that of a corresponding  $[y_n+H+Ag]^+$  ion resulting from cleavage of the same peptide bond are linked by the following relationship:  $[y_n+H+Ag]^+=[M+Ag]^++Ag^+-[b_n-H+Ag]^+$ . The observation of the corresponding  $[y_n+H+Ag]^+$  ions increases the confidence of the assignment for these peptides.

Please replace the paragraph beginning at p. 15, line 10 with the following paragraph:

The greatest potential for application of sequencing argentinated oligopeptides that we envisage is in the area of automated sequence tag analysis in proteomics, where accurate, automatic peak, and subsequently residue, assignment can be made because of the presence of four series of peaks, the  $[b_n+OH+Ag]^+$ ,  $[b_n-H+Ag]^+$ ,  $[a_n-H+Ag]^+$ , and  $[y_n+H+Ag]^+$  ions, and identification of only three or four residues are needed for often unambiguous searches (Shevchenko et al. (1996); Figey et al. (1996)). Indeed, inputting the partial sequences shown in FIG. 6, together with the bracketing  $m/z$  values of the  $b$  ions for the corresponding protonated product ions (for FIG. 6a, they are  $335.2+1-108.9=227.3$  and  $610.1+1-108.9=502.2$ ; thus the sequence tag search parameters are  $227.3/F/A/G/502.2$  for this tryptic peptide) plus the peptide mass (the  $m/z$  value of the precursor ion minus 108.9) and the molecular mass of the protein (the  $m/z$  value of the  $[M+^{109}Ag]^+$  ion of the protein prior to trypsin digestion minus 108.9, which was measured in a separate experiment and provided to the analyst) unambiguously identifies the protein as bovine ubiquitin after a search using Peptide Scan (PE SCIEX) with sequence index files (database) downloaded from the European Molecular Biology Laboratory <ftp://ftp.embl-heidelberg.de/pub/databases/nrdb/> (~~ftp://ftp.embl-heidelberg.de/pub/databases/nrdb/~~). The results are summarized in Table 4. All the proteins are ubiquitin; swiss/p02248, bovine ubiquitin, has the best match with the measured protein molecular mass ( $M_r$ ) of 8565 Da and has the correct sequence as subsequently revealed.